AD_____

Award Number: W81XWH-11-1-0406

TITLE: Development of a Tetrathioether (S4) Bifunctional Chelate System for Rh-105

PRINCIPAL INVESTIGATOR: Valerie Carroll

CONTRACTING ORGANIZATION: University of Missouri, Columbia, MO 65211

REPORT DATE: July 2012

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-07-2012	Annual Summary	15 JUN 2011 - 14 JUN 2012

4. TITLE AND SUBTITLE
Development of a Tetrathioether (S4) Bifunctional Chelate System for Rh-105

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0406

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Valerie Carroll, Don Wycoff, Fabio Galazzi, Timothy Hoffman, Silvia Jurisson

E-Mail: vnrvx4@mail.missouri.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University Of Missouri
Columbia MO 65211

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this project is to develop and evaluate a potential new molecule for site directed prostate cancer therapy. The moderate β^- emissions (0.566 MeV [70%], 0.248 MeV [19%], $t_{½}$= 35.4 h) of rhodium-105 are well suited for therapy of solid tumors. In addition to its favorable nuclear properties, the kinetic inertness of low-spin d^6 Rh(III) complexes make them good candidates for radiopharmaceutical use. The bifunctional chelate method will be utilized to couple rhodium-105 with a bombesin (BBN) targeting vector. Bombesin targets gastrin releasing peptide (GRP) receptors, which have been shown to be over-expressed on the surface of prostate cancer cells. Here we report the successful synthesis and characterization of a bombesin agonist coupled tetrathioether (S4) bifunctional chelate system (S4-BBN) and the resulting $[Rh(III)\text{-}S4\text{-}BBN]^+$ complex. Characterization of this previously unknown complex contributes to existing knowledge in the field of radiopharmaceutical chemistry and drug discovery. Studies now underway include *in vitro* determination of IC_{50} values employing the PC-3 human prostate cancer cell line and biological evaluation of the $[Rh(III)\text{-}S4\text{-}BBN]^+$ complex in animal xenograft models. If successful, this project will improve frontline treatment of patients with metastatic prostate cancer.

15. SUBJECT TERMS
Prostate Cancer, Radiotherapy, Radiopharmaceuticals, Rhodium-105, Tetrathioether, Bombesin

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	9	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction:

The purpose of this project is to synthesize a new radiotherapeutic agent [^{105}Rh-S4-BBN]$^{+}$ and to evaluate its potential for targeted prostate cancer therapy. If successful, the resulting radiopharmaceutical will improve frontline treatment of patients with metastatic prostate cancer. [^{105}Rh-S4-BBN]$^{+}$ can be administered in nanomolar concentrations potentially limiting pharmacologic effects to the patient and can be used in combination with existing therapies providing another tool for battling prostate cancer.

Body:

Specific Aim 1: Synthesis of bombesin (7-14) coupled tetrathioether bifunctional chelate

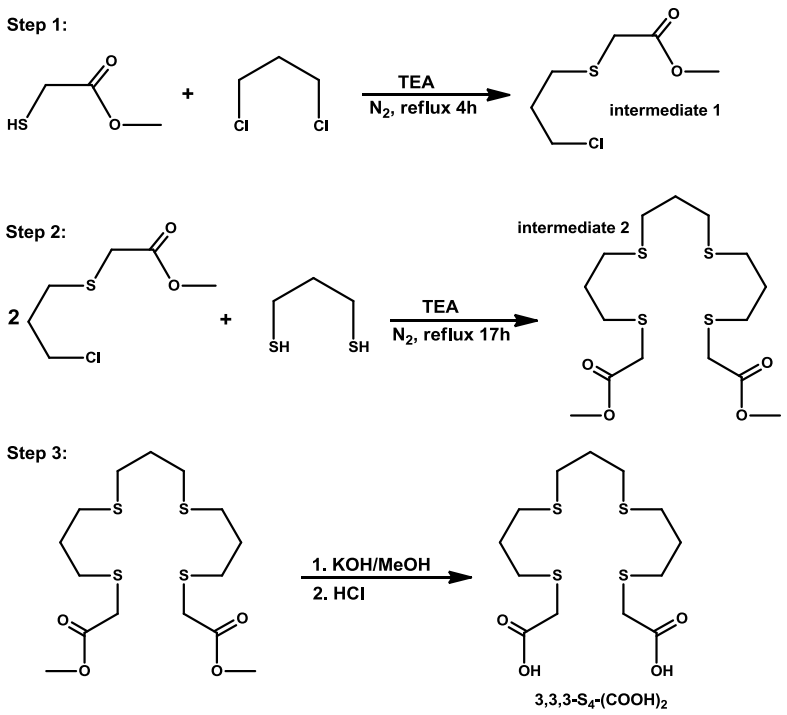
1a: Synthesize dicarboxylic acid functionalized ligand 3,3,3-S4-(COOH)$_2$

The dicarboxylic acid functionalized ligand 3,3,3-S4-(COOH)$_2$ was synthesized according to methods described in Goswami et al.[1] as shown in reaction Scheme 1. Methylthioglycolate (4.0 mL, 42 mmol) and an excess of 1,3-dichloropropane (40 mL, 410 mmol) were brought to reflux at 84^{o}C under nitrogen followed by the dropwise addition of 6.6 mL (47 mmol) of dry triethylamine (TEA). The triethylammonium chloride salt was removed by filtration and excess dichloropropane was removed under vacuum at 60^{o}C yielding a yellow oil. The separation of intermediate **1** using the vacuum distillation technique described in Goswami et al[1] was not sufficient and thus the oil was purified by silica gel column chromatography with a dry mass of silica gel to sample ratio

of 80:1. The sample was loaded with a 50% mixture of hexanes and diethyl ether and then eluted with the same solution. The fractions containing intermediate 1 were collected and the mobile phase was removed under nitrogen gas. The purified intermediate **1** recovered in 71% yield was characterized by NMR. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm: 3.75 (s, -O-C**H**$_3$, 3H), 3.65 (t, -C**H**$_2$-Cl, 2H), 3.24 (s, -C**H**$_2$-COO, 2H), 2.80 (t, -CH_2-C**H**$_2$-S, 2H), 2.07 (p, CH_2-C**H**$_2$-CH_2, 2H)

Two molar equivalents of intermediate **1** (3.58 g, 19.6 mmol) were combined with 2.8 mL (20 mmol) of dry triethylamine (TEA). 1,3-propane dithiol (0.96 mL) was added to the mixture dropwise, and the solution was refluxed under nitrogen at 94^{o}C over night for 17 hours. The mixture was then cooled and slurried with diethyl ether. Triethyl ammonium chloride was removed by filtration and diethyl ether removed under nitrogen. The intermediate **2** oil was purified by silica gel chromatography as described above. A yield of 60% was isolated. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm: 3.75 (s, -O-C**H**$_3$, 6H), 3.24 (s, -C**H**$_2$-COO, 4H), 2.75 (t, -C**H**$_2$-S-CH_2-COO, 4H), 2.62 (t, C**H**$_2$-S-C**H**$_2$, 8H), 1.87 (m, CH_2-C**H**$_2$-CH_2, 6H).

Intermediate **2** was heated to 90^{o}C in an alkaline solution of KOH (1.194 g) in 6 mL methanol for 55 min. The solid product was filtered, washed with methanol and dissolved in 10 mL of water. 6 M HCl was added to the solution dropwise resulting in the precipitation of the desired product 3,3,3-S4-$(COOH)_2$. The precipitate was filtered and dried under vacuum. A 71% yield of the final product was isolated, ^{1}H NMR (500 MHz, CD_3CN): δ ppm: 3.22 (s, -C**H**$_2$-COO, 4H), 2.69 (t, -C**H**$_2$-S-CH_2-COO, 4H), 2.58 (t, C**H**$_2$-S-C**H**$_2$, 8H), 1.81 (m, C**H**$_2$-S-C**H**$_2$, 8H)



Scheme 1: Synthesis of 3,3,3-S4-$(COOH)_2$.[1]

1b. Prepare bombesin (7-14) coupled 3,3,3-S4-BBN ligand system.

Fmoc-8Aoc-BBN(7-14) was prepared using automated solid phase Fmoc chemistry with an Omega AAPPTEC 396 peptide synthesizer on a Rink Amide resin support. Manual coupling was used to conjugate the tetrathioether chelate by adding 60 μmol of 8Aoc-BBN(7-14) on the resin support to a stirring solution containing 50 μL (300 μmol) of diisoproplyelthyamine (DIEA), 100 μL (130 μmol) of N-methyl-2-pyrrolidone (NMP), 65 mg (17 μmol) of 3,3,3-S4-$(COOH)_2$, 45.5 mg (120 μmol) of O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU), and 27 mg (200 μmol) of hydroxybenzoltriazole (HOBT). The solution was stirred at 60°C for 30 min, cooled and then filtered.

S4-8Aoc-BBN was cleaved from the resin support in a solution of 5% water, 5% triisopropyl silane (TIS), and 5% phenol in trifluoroacetic acid (TFA), filtered and precipitated in cold *t*-butyl ether. The desired product was HPLC purified using a Prep Nova-Pak HR C18 Waters column (6 μm, 7.8 x 300 mm, 60 Å). It should be noted that thiol containing scavengers must be omitted from the cleavage solution as they may interact with the chelate thioethers. LC-MS analysis confirms formation of the pure 3,3,3-S4-8Aoc-BBN with a retention time of 42.47 min and m/z of 1436.2 Da (calculated = 1435.68 Da) using a Kromasil C18 HPLC column (5 μm, 150 x 4.6 mm, 100 Å) and a Finnigan TSQ7000 mass spectrometer.

Specific Aim 2: Synthesize and characterize non-radioactive [Rh-S4-BBN]$^+$ complex.

S4-8Aoc-BBN (0.5 mg, 3.1 x 10^{-4} mmol) was dissolved in 5 mL of 4% acetonitrile/ethanol solution. The solution was brought to reflux at 90^{o}C and 40 μL of $RhCl_3$ $3H_2O$ (0.9 mg, 3.4 x 10^{-4} mol) in acetonitrile was added dropwise. The mixture was refluxed at 90^{o}C for 1 h, cooled and then lyophilized. The resulting pale yellow solid was analyzed using LC-MS and MALDI TOF MS. Both methods confirm the formation of a [RhCl-S4-8Aoc-BBN]$^+$ complex where the rhodium is bonded to each of the four sulfur atoms, one chloride atom and the carboxylate pendant group as evidenced by a m/z of 1571.4 Da (calculated = 1571.02 Da) for the [RhCl-S4-8Aoc-BBN]$^+$ and a m/z of 786.3 Da (calculated = 786.51 Da) for the protonated [RhCl-S4-8Aoc-BBN]$^{2+}$ species with a retention time of 20.1 min using a Kromasil C18 HPLC column (5 μm, 150 x 4.6 mm, 100 Å) with a Finnigan TSQ7000 mass spectrometer for LC-MS and an AB Sciex4700 mass spectrometer for MALDI TOF MS.

This complex was not the expected configuration $[RhCl_2\text{-S4-8Aoc-BBN}]^+$, however properties needed for a site directed bifunctional chelate system are satisfied. IC_{50} values employing the PC-3 human prostate cancer cell line will be determined for the $[RhCl\text{-S4-8Aoc-BBN}]^+$ complex.

Key Research Accomplishments:

- **Task 1.** Synthesized bombesin (7-14) coupled tetrathioether bifunctional chelate. **Completed.**
- **Task 2.** Synthesized and characterized non-radioactive $[Rh\text{-S4-BBN}]^+$ complex. **Completed.**
- **Task 3.** IC_{50} evaluation of the non-radioactive $[RhCl_2\text{-S4-BBN}]^+$ complex. **Currently underway.**
- **Task 4.** Radiolabeling of the S4-BBN chelate. **Currently underway.**

Reportable Outcomes:

- **Carroll, Valerie;** Wycoff, Donald; Sieckman, Gary; Hoffman, Timothy; Jurisson, Silvia; "Synthesis of a ^{105}Rh tetrathioether bombesin molecule for prostate cancer therapy" 243rd National Meeting of the American Chemical Society: San Diego, CA, March 25 – 29, 2012
- **Carroll, Valerie**; Demoin, Dustin; Hoffman, Timothy; Jurisson, Silvia; "Inorganic Chemistry in Nuclear Imaging and Radiotherapy: Current and Future Directions", *Radiochemica Acta,* Accepted June 6, 2012, to appear August/September 2012

Conclusion:

We have successfully synthesized a 3,3,3-S4-BBN bifunctional chelate system to selectively deliver rhodium-105 to prostate cancer tumor tissue, and the target molecule $[Rh\text{-S4-BBN}]^+Cl^-$ has been isolated and characterized on the macroscopic level. The

target molecule has been produced with sufficient purity for evaluation using *in vitro* methods with the PC-3 human prostate cancer cell line to determine its affinity for GRP receptors. Evaluation of the rhodium-105 analog in xenograft animal models for potential utility as a radiotherapeutic agent for prostate cancer is planned over the next few months.

Refrences:

1. N. Goswami, C. Higginbotham, W. Volkert, R. Alberto, W. Nef, S. Jurisson, Rhodium-105 Tetrathioether Complexes: Radiochemistry and Initial Biological Evaluation, *Nuclear Medicine & Biology*, 1999(26); 951-957

2. N. Goswami, R. Alberto, C. L. Barnes, S. Jurisson, Rhodium(III) Complexes with Acyclic Tetrathioether Ligands. Effects of Backbone Chain Length on the Conformation of the Rh(III) Complex, *Inorganic Chemistry*, 1996(35); 7546-7555